# PERFORMANCE EVALUATION OF INNO-LIPA® HPV GENOTYPING *EXTRA* II ON FIRST-VOID URINE

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## BACKGROUND/OBJECTIVE

Urinary self-sampling for HPV detection offers a non-invasive alternative to clinician-collected cervical samples and has the potential to increase accessibility to HPV testing, simplify HPV prevalence studies and vaccine efficacy studies. During collection, the first void of urine (FVU) contains mucus and debris from exfoliated cervicovaginal cells, and hence contains significantly more HPV than random or midstream urine[1]. The INNO-LiPA® HPV Genotyping *Extra* II assay allows identification of 32 HPV genotypes and excellent performance (e.g. high sensitivity) has been shown using cervical scrapes[2].

Our aim was to assess the performance of the INNO-LiPA® HPV Genotyping *Extra* II assay on FVU samples in comparison to cervical scrape samples. Assessment was done through evaluation of (1) the success rate of the assay on FVU samples, and (2) the diagnostic agreement between FVU and cervical scrape samples. Additionally, the reproducibility of the sample process flow on both cervical scrape and FVU samples was evaluated.

## METHODS

For the evaluation of the success rate and diagnostic agreement on the one hand and the reproducibility on the other hand, a set of 119 and a set of 22 paired FVU and cervical scrape samples were tested respectively. FVU samples were collected using a Colli-Pee™ device with UCM-preservative (Novosanis NV, Belgium) while cervical material was collected with L-shaped endo/esocervical swab (Copan) in 20 mL PreservCyt solution (Hologic). All samples were collected between 2017-2019 and stored at -20°C until analysis.

DNA from FVU and cervical samples was extracted using the QIAamp DNA mini kit (Art. Code 51304, modified protocol as described in IFU of INNO-LiPA® HPV Genotyping *Extra* II AMP Kit) and the QIAamp Media Elute kit (Art. Code 57414, modified protocol as described in IFU of INNO-LiPA® HPV Genotyping *Extra* II AMP Kit) respectively. The extracted DNA was amplified and hybridized with the INNO-LiPA® HPV Genotyping *Extra* II assays, according to the manufacturer's instructions.

To demonstrate the reproducibility of the sample process flow on cervical scrape and FVU samples, two different aliquots of each sample were tested at different timepoints, with an interval of 7 months, using different batches of extraction, amplification, and INNO-LiPA kits.

## RESULTS

### Success Rate And Diagnostic Agreement

Paired FVU and cervical scrape samples (n= 119) were evaluated to assess the success rate of the INNO-LiPA® HPV Genotyping *Extra* II assay on FVU samples and the diagnostic agreement between FVU and cervical scrape samples.

For all 119 UCM-preserved FVU samples tested, a valid INNO-LiPA result was obtained, resulting in an overall success rate of 100% (95 CI [97.8; 100.0]).

Figure 1 shows the comparison between the INNO-LiPA® HPV Genotyping *Extra* II outcome on FVU and cervical scrape samples. The results were grouped into 3 categories based on the degree of HPV genotype (GT) similarity between the two sample matrices; identical, partly identical, and non-identical genotyping results. Genotyping results are identical when the number and type of genotypes are the same between the matrices or when the number and type of highest-classified risk genotypes are the same between the matrices along with one or more additional lower-classified risk HPV GT(s) in one of the matrices. Identical genotyping results were observed in 72% (85/119) of the examined samples. Partly identical HPV GT results were obtained for 19% of the samples (23/119): at least one identical high-risk (HR) HPV GT was detected in both sample matrices, while additional HR (and possibly lower-classified risk) HPV GTs were detected in only one of the matrices. These partly similar HPV GT results were most common in samples from multiple-genotype infected individuals. Non-identical genotyping results have no common highest-classified risk HPV GT detected between the two matrices, which was the case in about 9% of the samples (11/119).

Diagnostic agreement was defined as the number of concordant results on the total number of samples. Concordance between LiPA cervical scrape and LiPA FVU result was defined as: at least one HR GT result corresponds in HR HPV single/mixed infections, at least one pHR GT result corresponds in pHR single/mixed infections, and at least one LR GT result corresponds in LR single/mixed infections. Both the 'identical' and 'partly identical' categories come under this definition.

Hence, the overall diagnostic agreement of INNO-LiPA® HPV Genotyping *Extra* II between FVU and cervical samples was 90.7%.

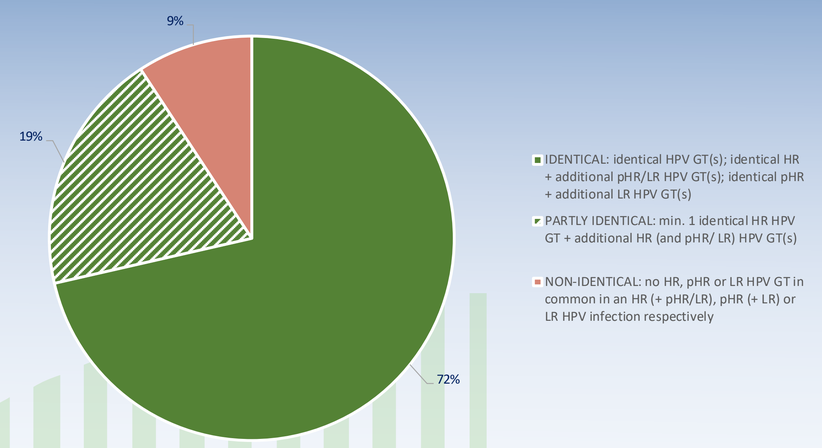


**Figure 1. HPV genotype comparison between cervical scrape and FVU samples.** Paired cervical scrape and FVU samples (n= 119) were collected and subjected to the INNO-LiPA® HPV Genotyping *Extra* II assay. Test results of both sample types were interpreted by using LiRAS for LiPA HPV v3.01 software and compared to each other. FVU: first void urine, HPV: Human Papilloma Virus, GT: genotype; HR: high-risk, pHR: possible high-risk, LR: low-risk

### Reproducibility

Reproducibility of the sample process flow was evaluated on 22 paired FVU and cervical scrape samples. The results are shown in Table 1. All samples except one showed concordant HPV GT results between the two timepoints. In case of FVU sample M018, HPV GT 66 was detected at the first timepoint while HPV GT 53 was observed in the corresponding cervical scrape sample; at the second timepoint HPV GT 53 was detected in both sample types. Retesting of the M018 FVU sample at timepoint 1 confirmed the detection of HPV GT 53 in the FVU sample.

Hence, reproducibility was demonstrated for the INNO-LiPA® HPV Genotyping *Extra* II process flow on both cervical scrape and FVU samples.

**Table 1: Reproducibility of the INNO-LiPA® HPV Genotyping *Extra* II process flow on FVU and cervical scrape samples**. Paired cervical scrape and FVU samples (n= 22) were subjected to the INNO-LiPA® HPV Genotyping *Extra* II assay at two timepoints with an interval of 7 months. Red: high-risk HPV genotype, orange: possible high-risk HPV genotype, green: low-risk HPV genotype.

| SAMPLE | FVU | | CERVICAL SCRAPE | |
|---|---|---|---|---|
| | T= X | T= X + 7 months | T= X | T= X + 7 months |
| M012 | 56, 59 | 56, 59 | 56, 59 | 56, 59 |
| M013 | 58, 68, 53 | 58, 68, 53 | 58, 59, 53 | 58, 53 |
| M016 | 68 | 68 | 68 | 68 |
| M018 | 66 / 53* | 53 | 53 | 53 |
| M023 | 16, 81 | 16, 81 | 16 | 16 |
| M028 | 66 | 66 | 66 | 66 |
| M030 | 31 | 31 | 18, 31 | 18, 31 |
| M031 | 18, 59, 6 | 18, 59, 6 | 18, 6 | 18, 6 |
| M033 | 16 | 16 | 16 | 16 |
| M035 | 45, 68 | 45, 68 | 45, 68 | 45, 68, 89 |
| M047 | 59 | 59 | 59 | 59 |
| M057 | 16, 31, 53 | 16, 33, 53 | 16, 53 | 16, 53 |
| M060 | 16, 51 | 16, 51 | 16, 51 | 16, 51 |
| M063 | 16 | 16 | 16 | 16 |
| M064 | 16 | 16 | 16 | 16 |
| M066 | 31, 66 | 31, 66 | 31 | 31 |
| M071 | 52 | 52 | 52 | 52 |
| M073 | 16 | 16 | 16 | 16 |
| M074 | 16, 89 | 16, 89 | 16 | 16 |
| M075 | 45 | 45 | 45 | 45 |
| M081 | 66 | 66 | 66 | 66 |
| M088 | 35, 82, 83 | 35, 82, 83 | 35 | 35 |

*: after retesting at timepoint 1, FVU sample M018 tested positive for HPV GT 53 instead of HPV GT66.

*References*

*1. Pathak, N. et al. (2014). Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. BMJ.*

*2. Lan Xu et al. (2018). Clinical evaluation of INNO-LiPA HPV genotyping Extra II assay using the VALGENT framework. Int. J. Mol. Sci.*

## CONCLUSION

Performance of the INNO-LiPA® HPV Genotyping *Extra* II assay using self-sampled FVU was evaluated and demonstrates the feasibility of the FVU sample application with high concordance of the HPV genotyping results for paired cervical samples. Additionally, the INNO-LiPA® HPV Genotyping *Extra* II process flow was demonstrated to be reproducible on both cervical scrape as FVU samples. Hence, non-invasive FVU self-sampling, in combination with the INNO-LiPA® HPV Genotyping *Extra* II assay, offers a promising alternative to clinician-collected cervical samples for HPV detection and genotyping.